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Drug release characteristics of unimolecular polymeric micelles

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Abstract

Biodegradable, unimolecular polymeric micelles possess several features that are attractive for drug delivery applications: Thermodynamic stability, ability to encapsulate and solubilize a hydrophobic guest molecule, biodegradability, as well as size and surface characteristics that prevent rapid clearance by the RES. Here we investigate the potential of these unimolecular polymeric micelles to release a drug for an extended time. Lidocaine was used as a model drug for in vitro studies using a horizontal diffusion cell and cellulose membrane that prevented polymer transport from the source to the receiver compartment. The transport of free lidocaine from source to receiver under sink conditions was zero-order and complete within 8 h. The transport of lidocaine initially encapsulated in polymer was zero-order for the first 14 h, and 96% of the lidocaine was detected within 24 h.

Keywords: Polymeric micelle; Poly(ethylene glycol); Biocompatible; Diffusion; Transport

1. Introduction

Micelles are used in drug delivery applications because they feature a lipophilic core that solubilizes hydrophobic molecules (e.g., drugs) and a hydrophilic shell that makes the entire assembly water-soluble. Applications have been investigated for parenteral or oral administration, but also for ophthalmic, topical, rectal and nasal delivery [1,2]. The ability of micelles to solubilize hydrophobic drugs expands the pharmaceutical potential of lipophilic drug molecules. In this capacity, micelles serve as drug reservoirs or 'microcontainers' that ultimately release drugs via diffusional processes.

A second important function of micelles is that their small size allows them to evade the body's screening mechanism, the reticuloendothelial system (RES). Recognition by the RES is known to be the main reason for removal of many drug delivery vehicles from the blood. RES recognition is considerably lowered for particles less than ca. 200 nm and with appropriately modified surfaces [3–10]. Similarly, the accumulation of particles within tumors is determined by their ability to penetrate through vascular pores [11] which is strongly dependent on their small size [12].

However, the formation and stability of micelles is both temperature- and concentration-dependent. Micelles are thermodynamically unstable at concentrations below their critical micelle concentration or

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'cmc'. For example, after micelles are injected into the bloodstream, the thermodynamic equilibrium between surfactants and micelles may cause serious toxicity problems due to potentially large fluctuations in drug concentrations accompanied by the breakdown in micellar structure into surfactant molecules. The dilution of micelles is particularly large after oral and intravenous administration, and can cause the unwanted precipitation of hydrophobic drugs [13].

Several groups have demonstrated that polymeric micelles, micelles based on amphiphilic block copolymers, have prolonged stability over conventional surfactant-based micelles. For example, block copolymers based on Pluronics, which are blocks of hydrophilic poly(ethylene oxide) (PEO) and hydrophobic poly(propylene oxide) (PPO), are being investigated by many groups as drug delivery systems [14,15]. These triblock copolymers form polymeric micelles in which drug molecules are solubilized in the inner hydrophobic PPO core, whereas the PEO blocks form the outer hydrophilic shell to induce water-solubility. For example, the neuroleptic action of haloperidol when delivered into mice as aqueous Pluronic solutions was increased relative to aqueous haloperidol solutions [16].

Diblock copolymers composed of poly(aspartic acid) or poly(β-benzyl-L-aspartate) as the hydrophobic block and poly(ethylene glycol) (PEG) as the hydrophilic part were also demonstrated to form polymeric micelles [17–19]. Other examples include poly(lactic acid)–PEG [20], poly(γ-benzyl-L-glutamate)–PEO [21–23], poly(ε-caprolactone)–PEG [24,25], and poly(β-benzyl-L-aspartate)–PEO [26,27].

As described above, several groups have demonstrated prolonged stability of polymeric micelle systems, yet these systems are still dynamic entities with a fluctuating size, and drug molecules can pass between the micelle and the aqueous solution [28]. The inherent thermodynamic instability of micelles can be overcome by construction of an assembly that topologically resembles micelle architecture but with the surfactant chains covalently bound together. Polymeric structures with a micellar topology were first described by Newkome et al. [29–31] as "unimolecular micelles"; these polymers consisted of lipophilic, aliphatic polymer chains with hydrophilic (ionic or non-ionic) chain ends and demonstrated micellar behavior but were nondegradable.

We recently reported a methodology to synthesize degradable, hyperbranched, amphiphilic polymers with micellar properties [32,33]. We define these novel polymers as "unimolecular polymeric micelles" because previous studies demonstrated that one (individual) molecule of polymer behaves as a single micellar entity [32,34]. In contrast to the block polymeric micelles, these unimolecular polymeric micelles do not have a cmc by definition. The polymers are synthesized from mucic acid (a sugar), alkyl chains (fatty acid-like units) and poly(ethylene glycol) (PEG). The structures are highly branched with a hydrophobic core of alkyl chains surrounded by a hydrophilic surface layer of PEG. Because PEG creates a steric and chemical barrier that prevents interaction with proteins or cells [35], long-term circulation in the blood is expected due to the avoidance of renal filtration and reduction in uptake by the liver [36].

The ability of these unimolecular polymeric micelles to encapsulate and solubilize drugs was previously explored [32,37]. In vitro studies established the proportional relationship between the core size of the unimolecular polymeric micelle structure and its encapsulation capacity, i.e., structures with larger cores can encapsulate a larger quantity of drug. These encapsulation studies were performed using high pressure liquid chromatography (HPLC) to monitor lidocaine, a hydrophobic drug that is used as a local anesthetic. The encapsulation number, defined as the number of molecules that can be entrapped within the amphiphilic polymers, increased as the lipophilicity of the interior increased. Within the matrix of polymers evaluated, Core(laur)PEG5 (Fig. 1) had the highest loading capacity of lidocaine while maintaining its water-solubility.

Based upon these earlier results, we evaluated the release characteristics of the Core(laur)PEG5 polymer system by monitoring lidocaine transport through a cellulose membrane. In this paper, we describe the diffusional release of lidocaine using Core(laur)PEG5 to demonstrate the feasibility of sustained release from unimolecular polymeric micelles. For convenience, the polymer was named to identify its components; Core(laur)PEG5 has an interior core consisting of the lauroyl ester ('laur') of

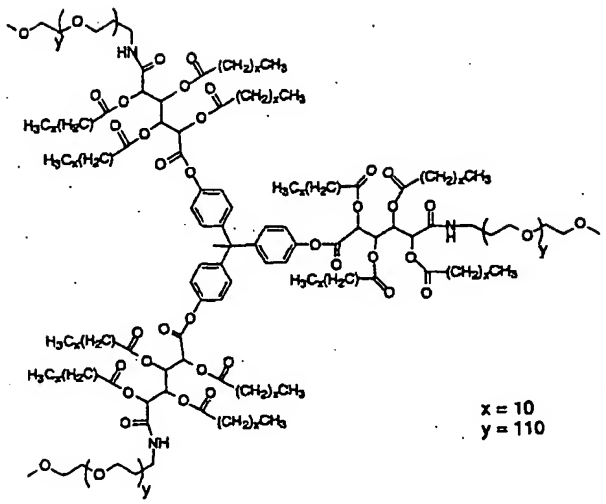

Fig. 1. Structure of unimolecular polymeric micelle, Core(laur)PEG5.

mucic acid and an exterior shell of methoxy-terminated PEG of molecular weight 5000.

2. Materials and methods

2.1. Materials

All common chemicals and lidocaine were purchased from Sigma/Aldrich (spectroscopic grade) and used without further purification. PEG with molecular weight of 5000 was purchased from Shearwater Polymers (Huntsville, AL). The polymer, Core(laur)PEG5 with molecular weight of 19 000 was synthesized according to previously described methods [32]. Spectra/Pore CE cellulose ester membrane was purchased from Spectrum Medical Industries (Laguna Hills, CA).

2.2. High pressure liquid chromatography conditions

Lidocaine concentration was quantitated by high pressure liquid chromatography (HPLC) according to a calibration curve generated from a series of standard lidocaine solutions ranging from 0.005 to 0.5 mg/ml lidocaine. The linearity of the curve indicated a direct, proportional relationship between absorbance and lidocaine concentration. Using the equation of the lidocaine calibration curve, the amount of lidocaine diffusionally released from the unimolecular polymeric micelle core was calculated. PEG was used as the HPLC control.

HPLC was performed on a Perkin Elmer Series 200 LC system equipped with a PE-CR C_{18} reverse phase column operated at room temperature, Applied Biosystems ultraviolet detector (254 nm), Series 200 LC pump and ISS 200 autosampler. Analytical data was collected into digital Celebris 466 computer through PE 900 series interface and PE 600 series link. The collected data was processed by PE Turbochrom 4 software. Methanol was used as mobile phase with a flow rate of 0.5 ml/min. Samples were dissolved and filtered using 0.45 μm PTFE syringe filters prior to column injection.

2.3. In vitro transport experiments

Transport experiments were performed with a Crown Glass two-reservoir diffusion cell system (Somerville, NJ) bound by a cellulose ester membrane with molecular weight cut-off (MWCO) of 10 000. Lidocaine was used as the drug. Water was used as the medium instead of buffer solution for ease of HPLC analysis. The diffusional release of lidocaine from Core(laur)PEG5 loaded with 1.6% lidocaine [32] was examined as a function of lidocaine transported through the cellulose membrane.

Sample solution (2.0 ml, at a concentration of 0.50 mg lidocaine/ml) was placed in one reservoir (source) while fresh distilled water (2.0 ml) was placed in the other reservoir (receiver) separated by the cellulose membrane. Sink conditions were maintained through the experiment. During the course of the experiment, solution in the receiver was removed at defined intervals and quantitated by HPLC. The receiver was filled with fresh distilled water (2 ml) immediately. Samples were taken at 1 h intervals over the course of 9 h. Cumulative release is expressed as total percent lidocaine that diffused from the polymer and was transported through the cellulose membrane over time.

As controls, several studies were carried out with aqueous solutions made from (1) free lidocaine, (2) lidocaine and PEG5, and (3) free lidocaine using polymer-treated cellulose membranes. For the third control experiment, the cellulose membranes were

treated as follows. After performing one experiment with free lidocaine (with no polymer) in the diffusion cell, the aqueous lidocaine solutions were removed from both the source and receiver sides of the diffusion cell without disassembling the cell. Distilled water (3.0 ml) was introduced to the receiver side, and 3.0 ml of aqueous polymer solution (50 mg/ml) introduced to the source side. Both sides were stirred using magnetic flea bars. The polymer solution contacted the source side of the cellulose membrane in this fashion for 48 h. Afterwards, the source and receiver side solutions were removed using a Pasteur pipette and each side rinsed twice by filling the compartment with distilled water (no mechanical agitation). The experiment with the lidocaine was then repeated using the same membrane just exposed to the polymer solution. For all controls, identical experimental conditions were used such as lidocaine concentration, polymer concentration, solution volume and temperature.

3. Model

A simple model was used to describe the release of the drug from the polymeric micelles. In developing the model, the polymeric micelle was envisioned as a hydrophobic core surrounded by a highly branched layer. This highly branched region becomes increasingly hydrophilic away from the core. It is likely that water penetrates into the hydrophilic branches, forming water-filled pore structures through which the drug may diffuse. The drug is loaded in the hydrophobic core at a very high concentration (region 1), and diffuses away from the core through a tortuous path toward the external aqueous surroundings (region 2). There is partitioning of drug at the interface between the polymer core and the branched region. The release of the drug from the polymeric micelle (region 1) can be expressed as:

$$V_1\frac{dC_1}{dt} = -K(m_1C_1 - C_2) \quad (1)$$

where V_1 is the volume of the micelles that are assumed to be spherical, K is a transport parameter, and m_1 represents the partition coefficient of the drug between the core and the interior of the branched polymer region. Because the lidocaine concentration in the core of the micelle is very high compared with its concentration in water, C_2 may be neglected in Eq. (1):

$$V_1\frac{dC_1}{dt} = -K_1C_1 \quad (2)$$

where K_1 is equal to Km_1. As the drug is released from the micelle into the source side solution, and then transported from the source side solution through the membrane to the receiver side (region 2), the source-side concentration may be described by:

$$V_2\frac{dC_2}{dt} = -K_1C_1 - k_2A_2(C_2 - C_3) \quad (3)$$

Because the receiver-side solution was frequently replaced in order to maintain a low concentration such that $C_3 \ll C_2$, C_3 may be neglected in Eq. (3). A_2 in Eq. (3) is the membrane area available for mass transfer, and k_2 is the mass transfer coefficient. Finally, the receiver-side concentration is described by:

$$V_3\frac{dC_3}{dt} = -k_2A_2C_2 \quad (4)$$

In Eq. (4), C_3 is the cumulative amount of drug that has been transported to the receiver-side, divided by the volume of the receiver-side. The initial concentration of drug in the micelle was known, and the concentrations C_2 and C_3 initially are zero. Eqs. (2)–(4) were solved simultaneously subject to these initial conditions using Polymath for Windows version 5.0 to provide values for C_1, C_2, and C_3 as functions of time.

4. Results and discussion

Unlike the nanoparticles created from block copolymer micelles [25,38], the unimolecular polymeric micelles are completely water-soluble such that polymer solutions are completely transparent at all concentrations. Because of the high water-solubility of our polymers, the conventional methods for evaluating drug release (e.g., centrifugation) are not applicable. Therefore, a method based on an artificial barrier provided by dialysis tubing was developed using HPLC. A two-reservoir diffusion cell system

bounded by a cellulose ester membrane with molecular weight cut-off (MWCO) of 10 000 was utilized. The cellulose ester membrane is the same membrane used in dialysis tubing, which is frequently used to monitor diffusion [15,22,25]. There are several advantages to using the diffusion cell system over dialysis tubing [39,40]: (i) Only a small piece of dialysis tubing (1 mm diameter) is needed as the separating membrane, (ii) small quantities of solution are required (3.0 ml), and (iii) both the source and receiving solutions can be readily sampled during the course of the experiment.

Based on control experiments, the membrane allowed small molecules such as lidocaine to permeate readily but prevented the transport of high molecular weight species (e.g., PEG with M_w of 5000). A diffusional release study of lidocaine from Core(laur)PEG5 that showed the highest loading efficiency [32] was performed. The source solution was made from polymer loaded with lidocaine (1.6 wt%) in distilled water.

To verify the diffusional release process of polymer-encapsulated drug (i.e., lidocaine), two control experiments were carried out under the same conditions: free lidocaine in water was one control, the second control monitored the transport of lidocaine from a PEG-containing solution. There was no statistical difference between the profiles of free lidocaine (first control) and lidocaine in the presence of PEG (second control). In these two experiments, the profiles were zero-order and all the free lidocaine was transported through the membranes into the receiver side within 8 h.

In contrast, when lidocaine was loaded onto Core(laur)PEG5, lidocaine was slowly released from the polymer over a 24 h period (Fig. 2).

For the polymer-lidocaine release experiment using Core(laur)PEG5, the process was zero-order for the first 14 h, but the release of lidocaine was sustained for 24 h. After 24 h, 96% of the lidocaine originally encapsulated in the polymer had been released and transported to the receiver side. In contrast, 96% of the lidocaine was observed in the receiver side by 7 h in the control experiments. The experiment utilizing polymer-encapsulated lidocaine was extended to 50 h, but no more lidocaine was detected in this extended time period. The slope of the zero-order release portion of the release profile

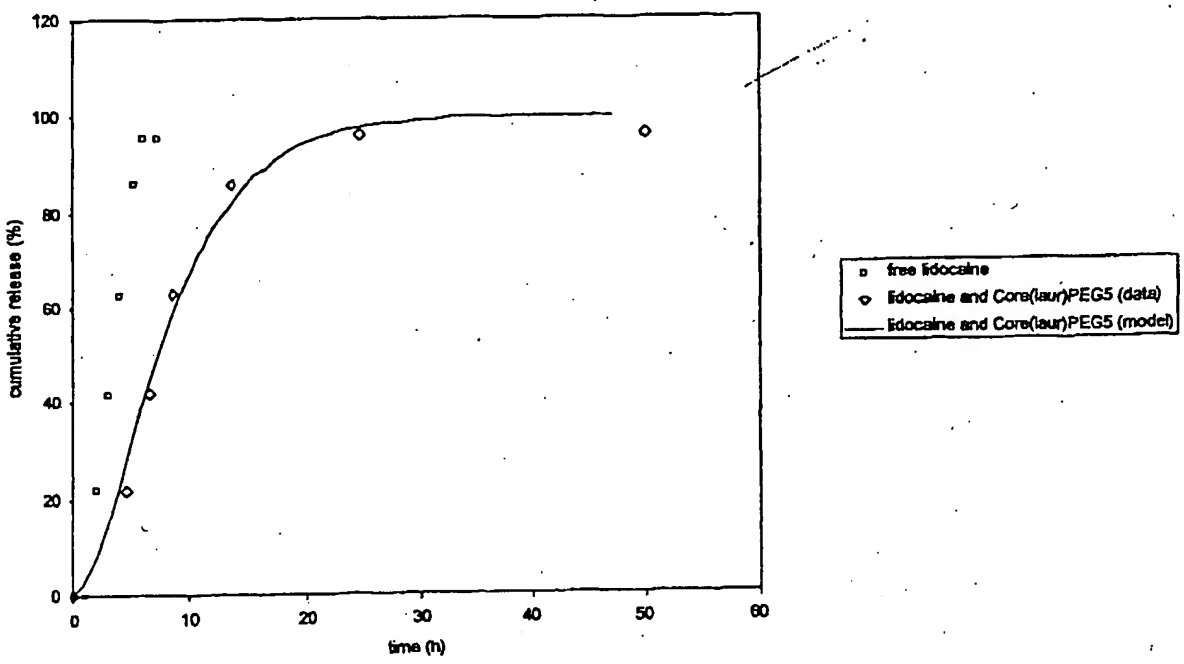

Fig. 2. Cumulative release of free lidocaine (open squares) and polymer-encapsulated lidocaine (open diamonds). The solid curve shows polymer encapsulated lidocaine release as described by the model.

for the free lidocaine control is 14.0 wt%/h; whereas, the slope for the initial region of the polymer-bound lidocaine curve is only 7.0 wt%/h. Although the release rate of lidocaine from the polymer is not measured directly, we can infer from these results that sustained release occurs because the transport rate is slower when lidocaine is encapsulated within the unimolecular polymeric micelle.

The mathematical model developed in Eqs. (1)–(4) was used to describe the release of the encapsulated drug in the diffusion cell system. The cumulative release described by the model is shown in Fig. 2. The model is not intended to elucidate the mechanism of release of the encapsulated drug from the polymer, but does account for the contribution of the unimolecular polymeric micelle toward the sustaining the release of the encapsulated drug longer than that of the free drug. The value of the mass transfer coefficient k_2 was determined by regression of the following equation to fit the release data for free lidocaine:

$$C_3 = C_3^0[1 - \exp(-k_2 A_2 t/V_2)] \quad (5)$$

Eq. (5) is a general expression for the receiver side concentration as described previously by Flynn et al. [41]. The parameter K_1 was determined from the experimental release data for the encapsulated lidocaine. The values of K_1 and k_2 are 7.85×10^{-3} cm^3/s and 5.32×10^{-5} cm/s, respectively.

To clarify a concern that slowed release might be a function of the polymer clogging the membrane pores, a third control experiment was performed as follows. First, the transport of free lidocaine through the cellulose membrane was measured. Then the lidocaine solution was removed and replaced with a solution containing unimolecular polymeric micelle. After 48 h, the polymer solution was removed, replaced with free lidocaine solution, and the transport of free lidocaine measured again. Transport of lidocaine through the polymer-treated membrane was identical to control experiments using solutions of free lidocaine (see related curve of Fig. 2, open squares). With this third control experiment, we conclude that sustained release is a result of the hydrophobic–hydrophobic interactions between the drug and the hydrophobic core of the polymer.

5. Conclusion

The potential for unimolecular polymeric micelles to encapsulate and deliver a drug in a sustained manner was established. Although the release rate of the drug from the unimolecular polymeric micelle was not measured directly, the results indicate that lidocaine encapsulated in the polymer is transported at a much slower rate than free lidocaine. The zero-order portion of the release profile was two times slower for encapsulated lidocaine than for free lidocaine, while the duration of the release was three times longer for polymer-encapsulated lidocaine. This data suggests that sustained release of lidocaine occurs as lidocaine initially encapsulated in the polymer core diffuses from the polymer structure. This phenomenon is likely due to hydrophobic–hydrophobic interactions between the drug (lidocaine) and the polymer core, which slows the diffusion of lidocaine from the polymer.

Ultimately, the small size (ca. 50 nm) of these small, unimolecular polymeric micelles [32,33] may lead to passive targeting drug delivery systems for certain tumors. The sustained release of anti-tumor drugs is the focus of future work because these polymeric micelles demonstrate hydrolytic stability [42] and biocompatibility [43].

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